



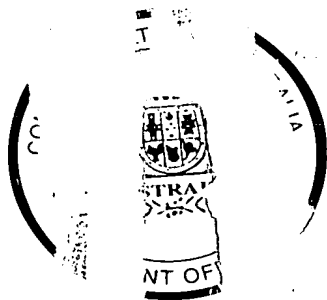
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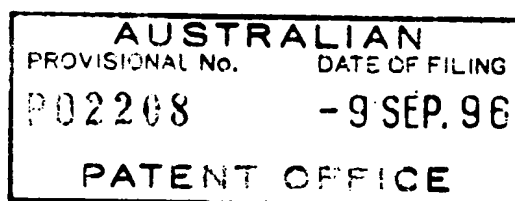
I, SMILJA DRAGOSAVLJEVIC, TEAM LEADER EXAMINATION
SUPPORT AND SALES hereby certify that annexed is a true copy of the
Provisional specification in connection with Application No. PO 2208 for a
patent by THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL
RESEARCH as filed on 09 September 1996.

WITNESS my hand this
Tenth day of March 2003

S. Dragosavljevic

SMILJA DRAGOSAVLJEVIC
TEAM LEADER EXAMINATION
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THE WALTER AND ELIZA HALL
INSTITUTE OF MEDICAL RESEARCH

A U S T R A L I A
Patents Act 1990

PROVISIONAL SPECIFICATION
for the invention entitled:

"A NOVEL HAEMOPOIETIN RECEPTOR AND GENETIC SEQUENCES
ENCODING SAME - III"

The invention is described in the following statement:

- 1A-

A NOVEL HAEMOPOIETIN RECEPTOR AND GENETIC SEQUENCES ENCODING SAME - III

5 The present invention relates generally to a novel haemopoietin receptor or components or parts thereof and to genetic sequences encoding same. The receptor molecules and their components and/or parts and the genetic sequences encoding same of the present invention are useful in the development of a wide range of agonists, antagonists, therapeutics and diagnostic reagents based on ligand interaction with its receptor.

10

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

15

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

20

The rapidly increasing sophistication of recombinant DNA techniques is greatly facilitating research into the medical and allied health fields. Cytokine research is of particular importance, especially as these molecules regulate the proliferation, differentiation and function of a wide variety of cells. Administration of recombinant cytokines or regulating cytokine function
25 and/or synthesis is becoming increasingly the focus of medical research into the treatment of a range of disease conditions.

Despite the discovery of a range of cytokines and other secreted regulators of cell function, comparatively few cytokines are directly used or targeted in therapeutic regimens. One reason
30 for this is the pleiotropic nature of many cytokines. For example, interleukin (IL)-11 is a

- 2 -

functionally pleiotropic molecule (1,2), initially characterized by its ability to stimulate proliferation of the IL-6-dependent plasmacytoma cell line, T11 65 (3). Other biological actions of IL-11 include induction of multipotential haemopoietin progenitor cell proliferation (4,5,6), enhancement of megakaryocyte and platelet formation (7,8,9,10), stimulation of acute
5 phase protein synthesis (11) and inhibition of adipocyte lipoprotein lipase activity (12, 13).

Interleukin-13 (IL-13) is another important cytokine which shares a number of structural characteristics with interleukin-4 (IL-4) [reviewed in 14 and 15]. The genes for IL-4 and IL-13 have a related intron/exon structure and are located close together on chromosome 5 in the
10 human and the syntenic region of chromosome 11 in the mouse (14, 15). At the protein level, IL-4 and IL-13 share approximately 30% amino acid identity, including four cysteine residues. Biologically, IL-13 and IL-4 are also similar, being produced by activated T-cells and acting upon macrophages to induce differentiation and suppress the production of inflammatory cytokines. Additionally, human IL-13 may act as a co-stimulatory signal for B-cell
15 proliferation and affect immunoglobulin isotype switching (14, 15). The diverse and pleiotropic function of IL-13 and other haemopoietic cytokine makes this molecule an important group to study, especially at the level of interaction of the cytokine with its receptors. Manipulation and control of cytokine receptors and of cytokine-receptor interaction is potentially very important in many therapeutic situations, especially where the target cytokine
20 is functionally pleiotropic and it is desired to block certain functions of a target cytokine but not all functions.

Research into IL-13 and its receptor has been hampered due to the inability to clone genetic sequences encoding all or part of the IL-13 receptor. In accordance with the present invention,
25 genetic sequences have now been cloned encoding the IL-13 receptor α -chain. The availability of these genetic sequences permits the development of a range of therapeutic and diagnostic agents capable of modulating IL-13 activity as well as the activity of cytokines related at the level of IL-13 receptor structure.

- 3 -

Accordingly, one aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an haemopoietin receptor α -chain from an animal or a component, fragment, part, derivative, homologue or analogue thereof.

5

More particularly, the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding the IL-13 receptor α -chain from an animal or a component, fragment, part, derivative, homologue or analogue thereof.

10

In a related embodiment, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding the IL-4 receptor α -chain from an animal or a component, fragment, part, derivative, homologue or analogue thereof.

15

Preferably, the animal is a mammal or a species of bird. Particularly, preferred mammals include humans, laboratory test animals (e.g. mice, rabbits, guinea pigs), livestock animals (e.g. sheep, horse, pigs, cows), companion animals (e.g. dogs, cats) or captive wild animals (e.g. kangaroos). Although the present invention is exemplified with respect to mice and humans,
20 the scope of the subject invention extends to all animals and birds.

The present invention is predicated in part on an ability to identify members of the haemopoietin receptor family on the basis of sequence similarity. Based on this approach, a genetic sequence was identified in accordance with the present invention which encodes the IL-
25 13 α -chain. The expressed genetic sequence is referred to herein as "NR4". NR4 has an apparent molecular weight when synthesised by transfected COS cells of from about 50,000 to about 70,000 daltons, and more preferably from about 55,000 to about 65,000 daltons. NR4 binds to IL-13 with low affinity and is considered, therefore, to be IL-13 receptor α -chain. Accordingly, the terms "NR4" and "IL-13 receptor α -chain" (or "IL-13 R α ") are used
30 interchangeably throughout the subject specification. Furthermore, in accordance with the

- 4 -

present invention, IL-13 binding to its receptor has been found to be competitively inhibited by IL-4 or a component thereof which may provide a method for controlling IL-13-receptor interaction and which may also provide a basis for the preparation and construction of mimetics.

5

Another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding IL-13 receptor α -chain having an amino acid sequence as set forth in SEQ ID NO:2 or having at least about 50% similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%, more preferably at least about 70%, even more
10 preferably at least about 80-85% and still even more preferably at least about 90-95% or greater.

A further embodiment of the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding the IL-13 receptor α -chain and having a
15 nucleotide sequence substantially as set forth in SEQ ID NO:1 or having at least about 50% similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%, more preferably at least about 70%, even more preferably at least about 80-85% and still even more preferably at least about 90-95% or greater.

20 Still another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding IL-13 receptor α -chain having an amino acid sequence as set forth in SEQ ID NO:4 or having at least about 50% similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%, more preferably at least about 70%, even more preferably at least about 80-85% and still even more preferably at least about 90-95% or
25 greater.

Yet still a further embodiment of the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding the IL-13 receptor α -chain and having a nucleotide sequence substantially as set forth in SEQ ID NO:3 or having at least about 50%
30 similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%,

- 5 -

more preferably at least about 70%, even more preferably at least about 80-85% and still even more preferably at least about 90-95% or greater.

Accordingly, the present invention extends to the sequence of nucleotides set forth in SEQ ID NO:1 or 3 or the sequence of amino acids set forth in SEQ ID NO:2 or 4 or single or multiple nucleotide or amino acid substitutions, deletions and/or additions thereto.

The present invention further extends to nucleic acid molecules capable of hybridising under low stringency conditions to the nucleotide sequence set forth in SEQ ID NO:1 or 3 or a complementary form thereof.

For the purposes of defining the level of stringency, reference can conveniently be made to Maniatis *et al* (1982) at pages 387-389 which are incorporated herein by reference where the washing step at paragraph 11 is considered herein to be high stringency. A low stringency wash is defined herein to be 0.1-0.2xSSC, 0.1% w/v SDS at 55-65°C for 20 minutes and a medium level of stringency is considered herein to be 2xSSC, 0.1% w/v SSC at $\geq 45^{\circ}\text{C}$ for 20 minutes. The alternative conditions are applicable depending on concentration, purity and source of nucleic acid molecules.

Yet another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a sequence which encodes an IL-13 receptor α -chain, said nucleic acid molecule having a nucleotide sequence substantially as set forth in SEQ ID NO:1 or 3 or a nucleic acid molecule which encodes a structurally similar IL-13 receptor α -chain or a derivative thereof and which is capable of hybridising to the nucleotide sequence substantially as set forth in SEQ ID NO:1 or 3 or a complementary form thereof under low stringency conditions.

Still yet another aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a sequence which encodes the IL-13 receptor α -chain having an amino acid sequence substantially as set forth in

- 6 -

SEQ ID NO:2 or 4 or comprises a nucleotide sequence coding for an amino acid sequence having at least about 50% similarity to the sequence set forth in SEQ ID NO:2 or 4 and is capable of hybridising to the sequence set forth in SEQ ID NO:1 or 3 under low stringency conditions.

5

The nucleic acid molecules contemplated by the present invention are generally in isolated form and are preferably cDNA or genomic DNA molecules. In a particularly preferred embodiment, the nucleic acid molecules are in vectors and most preferably expression vectors to enable expression in a suitable host cell. Particularly useful host cells include prokaryotic cells,
10 mammalian cells, yeast cells and insect cells. The cells may also be in the form of a cell line.

According to this aspect of the present invention there is provided an expression vector comprising a nucleic acid molecule encoding the IL-13 receptor α -chain as hereinbefore described, said expression vector capable of expression in a particularly host cell.

15

Another aspect of the present invention contemplates a recombinant polypeptide comprising a sequence of amino acids substantially as set forth in SEQ ID NO:2 or 4 or having at least about 50% similarity to all or part thereof. Preferably, the percentage similarity is at least about 60%, more preferably at least about 70%, even more preferably at least about 80-85% and still
20 even more preferably at least about 90-95% or greater.

The recombinant polypeptide contemplated by the present invention includes, therefore, components, parts, fragments, derivatives, homologues or analogues of the IL-13 receptor α -chain and is preferably encoded by a nucleotide sequence substantially set forth in SEQ ID
25 NO:1 or 3 or a molecule having at least about 50% similarity to all or part thereof or a molecule capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:1 or 3 or a complementary form thereof. The recombinant molecule may be glycosylated or non-glycosylated. When in glycosylated form, the glycosylation may be substantially the same as naturally occurring IL-13 receptor α -chain or may be a modified form of glycosylation.
30 Altered or differential glycosylation states may or may not affect binding activity of the IL-13

- 7 -

receptor α -chain.

The recombinant IL-13 receptor α -chain may be in soluble form or may be expressed on a cell surface or conjugated or fused to a solid support or another molecule.

5

The present invention extends to chemical analogues of the recombinant IL-13 receptor α -chain.

Chemical analogues of the recombinant IL-13 receptor α -chain contemplated herein include,
10 but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide synthesis and the use of crosslinkers and other methods which impose conformational constraints on the peptides or their analogues.

Examples of side chain modifications contemplated by the present invention include
15 modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH_4 ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6, trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5'-
20 phosphate followed by reduction with NaBH_4 .

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

25 The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed
30 disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other

- 8 -

substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

- 5 Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.
- 10 Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, 15 ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids.

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional 20 crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with $n=1$ to $n=6$, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α 25 and N_α -methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

- 9 -

Chemical modification of the recombinant IL-13 receptor α -chain may be important, for example, to increase serum half-life, to protect the molecule from enzymatic degradation and/or for diagnostic purposes.

- 5 The recombinant IL-13 receptor α -chain contemplated by the present invention is useful in the development of a range of agonists and antagonists of IL-13-receptor interaction. The recombinant molecule may also be used in the development of diagnostic agents.

Particularly useful agents encompassed by this aspect of the present invention are antibodies
10 to the recombinant IL-13 receptor α -chain. The antibodies may be monoclonal or polyclonal and are particularly useful as antagonists of IL-13-receptor binding or as diagnostic agents to qualitate or quantitate the presence of the IL-13 receptor α -chain. These antibodies may also be useful in the screening of similar components in other receptors such as IL-4 receptors.

- 15 Other agonists and antagonists include chemical molecules which, for example, structurally, functionally or electrochemically mimic or have similarities to IL-13 receptor α -chain or which comprise a solubilised form of the IL-13 receptor α -chain.

Such agents are useful in modulating IL-13-receptor interaction and these are useful in
20 enhancing or diminishing IL-13 related activities. This may be particularly important for cancers or tumours involving or resulting from excess IL-13 or from aberrant IL-13 molecules or to promote IL-13 function in the treatment of a range of conditions such as, but not limited to, immune deficiency.

- 25 The present invention further contemplates ribozyme and antisense molecules useful in reducing IL-13 receptor α -chain expression.

The present invention encompasses, therefore, pharmaceutical and diagnostic compositions comprising recombinant IL-13 receptor α -chain or parts thereof, antibodies thereto, agonists
30 or antagonists thereof or genetic molecules such as ribozymes, antisense molecules and

- 10 -

constructs useful in co-suppression.

The present invention is further described by the following non-limiting Figures and Examples.

5 In the Figures:

Figure 1 is a representation of the nucleotide [SEQ ID NO:1] and predicted amino acid [SEQ ID NO:2] sequence of murine NR4. The untranslated region is shown in lower case and the translated region in upper case. The conventional one-letter code for amino acids is employed, 10 potential asparagine linked glycosylation sites are underlined and the conserved cysteine residues and WSXWS motif of haemopoietin receptor family members are shown in bold. The predicted signal sequence is underlined in bold while the transmembrane domain is underlined with dashes. The sequence shown is a composite derived from the analysis of 8 cDNA clones derived from 3 libraries. The 5'-end of the sequence (nucleotides -60 to 351) is derived from 15 a single cDNA clone but is also present in genomic DNA clones that have been isolated.

Figure 2 is a photographic representation showing northern analysis of murine NR4 mRNA expression in selected tissues and organs.

20 **Figure 3** is a graphical representation depicting saturation isotherms of ^{125}I -IL-13 and ^{125}I -IL-4 binding; saturation isotherms depicted as Scatchard plots of IL-4 (◊) and IL-13 (●) binding to (A) COS cells expressing the IL-13R α (NR4), (B) CTLL cells and (C) CTLL cells expressing the IL-13R α (NR4). Data have been normalised to 1×10^4 COS cells and 1×10^6 CTLL cells and binding was carried out on ice for 2 to 4 hours.

25

Figure 4 is a graphical representation showing specificity of IL-4 and IL-13 binding; the ability of IL-4 (◊) and IL-13 (●) to compete for ^{125}I -IL-13 binding to (A) COS cells expressing the IL-13R α (NR4) and (C) CTLL cells expressing the IL-13R α (NR4) or to compete for IL-4 binding to (B) CTLL cells and (D). CTLL cells expressing the IL-13R α 30 (NR4) binding was carried out on ice for 2 to 4 hours and the data have been expressed as a

- 11 -

percentage of the specific binding observed in the absence of a competitor (■).

Figure 5 is a graphical representation showing factor dependent proliferation of cells expressing NR4. Two hundred (A) CTLL cells or (B) CTLL cells expressing the IL-13R α (NR4) were incubated in the absence of cytokine (■) or with various concentrations of IL-2 (□), IL-4 (◊) or IL-13 (●). After 48 hours viable cells were counted and data was expressed as a percentage of the number of viable cells observed with a maximal concentration of IL-2.

Figure 6 is a photographic representation showing cross-species conservation of NR4 (IL-13R α) gene.

Figure 7 is a representation of the nucleotide and corresponding amino acid sequence of murine and human NR4 (IL-13R α) genes. The nucleotide and predicted amino acid sequence of human (H) and murine (M) IL-13R α (NR4) were aligned by eye, with gaps (-) inserted to optimise the alignment. The numbering is for the murine clone, nucleotides that form part of the coding region are shown in upper case, whilst those of the untranslated regions are shown in lower case. Amino acids identical between the predicted murine and human proteins are indicated by (*). DNA encoding the murine signal sequence is underlined, with A26 or T27 being the predicted first amino acid of the mature protein.

Figure 8 is a photographic representation showing NR4 expression in mouse tissues.

Figure 9 is a photographic representation showing ^{125}I -IL-13 cross-linking to soluble NR4. Lane: ^{125}I -IL-13 (100,000 cpm) + 2 $\mu\text{g/ml}$ soluble NR4; Lane 2: ^{125}I -IL-13 (100,000 cpm) + 2 $\mu\text{g/ml}$ soluble NR4 in the presence of excess unlabelled IL-13; Lane 3: ^{125}I -IL-13 (100,000 cpm) + 2 $\mu\text{g/ml}$ soluble NR4 in the presence of excess unlabelled IL-4.

Figure 10 is a photographic representation of immunoprecipitation by anti-NR4 polyclonal antisera of cross-linked ^{125}I -IL-13 with IL-13R α (NR4). Lanes 9-11: soluble IL-13R α (30 μl of 3 $\mu\text{g/ml}$) cross-linked to ^{125}I -IL-13 (750,000 cpm) and immunoprecipitated with control

- 12 -

rabbit serum, or with anti-NR4 polyclonal antiserum in the presence or absence of 100 $\mu\text{g/ml}$ FLAG peptide, respectively; Lanes 12-14: soluble IL-13R α (NR4) (30 μl of 3 $\mu\text{g/ml}$) cross-linked to ^{125}I -IL-13 (750,000 cpm) in the presence of 0.5 $\mu\text{g/ml}$ unlabelled IL-13 and immunoprecipitated with an anti-IL-13R α (NR4) polyclonal antiserum in the presence or
5 absence of 100 $\mu\text{g/ml}$ FLAG peptide, respectively.

- 13 -

The following single and three letter abbreviations for amino acid residues are used in the specification:

5	Amino Acid	Three-letter Abbreviation	One-letter Symbol
	Alanine	Ala	A
	Arginine	Arg	R
10	Asparagine	Asn	N
	Aspartic acid	Asp	D
	Cysteine	Cys	C
	Glutamine	Gln	Q
	Glutamic acid	Glu	E
15	Glycine	Gly	G
	Histidine	His	H
	Isoleucine	Ile	I
	Leucine	Leu	L
	Lysine	Lys	K
20	Methionine	Met	M
	Phenylalanine	Phe	F
	Proline	Pro	P
	Serine	Ser	S
	Threonine	Thr	T
25	Tryptophan	Trp	W
	Tyrosine	Tyr	Y
	Valine	Val	V
	Any residue	Xaa	X

- 14 -

EXAMPLE 1

Isolation of genomic and cDNAs encoding NR4

ApoI digested genomic DNA, extracted from an embryonal stem cell line, was cloned into the
5 λ ZAPII bacteriophage (Stratagene, LaJolla, CA). Approximately 10^6 plaques from this library
were screened with a ^{32}P -labelled oligonucleotide corresponding to the sequence Trp-Ser-Asp-
Trp-Ser [SEQ ID NO:3] (16). Positively hybridising clones were sequenced using an
automated DNA sequencer according to the manufacturer's instructions (Applied Biosystems,
Foster City, CA). One clone appeared to encode for part of a new member of the haemopoietin
10 receptor family. Oligonucleotides were designed on the basis of this genomic DNA sequence
and were used in the conventional manner to isolate clones from mouse peritoneal macrophage
(Clontech Laboratories, Palo Alto, CA), mouse skin, mouse lung, mouse kidney, and WEHI-3B
(Stratagene, LaJolla, CA) λ -bacteriophage cDNA libraries.

15

EXAMPLE 2

Construction of expression vectors and transfection of cells

Using PCR, a derivative of the NR4 cDNA was generated which encoded for the IL-3 signal
sequence and an N-terminal FLAG epitope-tag preceding the mature coding region of NR4
(Thr27 to Pro424; Figure 1). The PCR product was cloned into the mammalian expression
20 vector pEF-BOS (17). Constructs were sequenced in their entirety prior to use. Cells were
transfected and selected as previously described (16, 18).

EXAMPLE 3

Northern blots

25 Northern blots were performed as previously described (16). The source of hybridisation
probes was as follows: NR4 - a PCR product from nucleotide 32 to 984 (Figure 1) and GAPDH
- a cDNA fragment spanning nucleotides (19) [REF REQUIRED].

- 15 -

EXAMPLE 4

Cytokines and experiments using radioiodinated cytokines

IL-2, IL-4, IL-7, IL-9, IL-13 and IL-15 were obtained commercially (R & D Systems, Minneapolis MN). For radioiodination, cytokines were dissolved at a concentration of 100 µg/ml in 10 mM sodium phosphate, 150 mM NaCl (PBS), 0.02% v/v Tween 20 and 0.02% w/v sodium azide at pH 7.4. An amount of 2 µg of IL-13 was radioiodinated using the iodine monochloride method (20, 21), while 2 µg of IL-4 was radiolabelled using diiodo-Bolton-Hunter reagent (16). Binding studies and determination of the specific radioactivity and bindability of labelled cytokines were performed as previously described (2).

For cross-linking experiments, recombinant murine IL-13 was produced as a FLAG-tagged protein in *Pichia pastoris*.

For cross-linking assays, aliquots of purified soluble IL-13R α (NR4) were incubated with ¹²⁵I-IL-13 in the presence or absence of a competitor in a final volume of 20 µl for at least 30 min at 40°C. Then 5 µl of a 12 mM solution of BS³ (Bis (Sulfosuccimidyl) suberate) in PBS containing 0.02% v/v Tween-20 was added and the mixtures were incubated for 30 min at 4°C. Samples were mixed with 8 µl of 4XSDS sample buffer and analysed by 13% w/v SDS-PAGE under non-reducing conditions. Gels were dried and visualised by either autoradiography or with a PhosphoImager.

EXAMPLE 5

Proliferation Assays

The proliferation of Ba/F3 and CTLL cells in response to cytokines was measured in Lux 60 microwell HL-A plates (Nunc Inc. IL, USA). Cells were washed three times in DME containing 20% v/v new born calf serum and resuspended at a concentration of 2 x 10⁴ cells per ml in the same medium. Aliquots of 10 µl of the cell suspension were placed in the culture wells with 5 µl of various concentrations of purified recombinant cytokines. After 2 days of incubation at 37°C in a fully humidified incubator containing 10% v/v CO₂ in air, viable cells

- 16 -

were counted using an inverted microscope.

EXAMPLE 6

Cloning and Characterisation of Murine NR4

5 A library was constructed in λ ZAPII using *ApoI* digested genomic DNA from embryonal stem cells and screened with a pool of ^{32}P -labelled oligonucleotides encoding the amino acid sequence Trp-Ser-Asp-Trp-Ser [SEQ ID NO:3] found in many members of the haemopoietin receptor family. One hybridising bacteriophage was found to contain a genomic clone that appeared to encode part of a novel member of the haemopoietin receptor family. This receptor
10 was given the operational name NR4. The sequence of the genomic clone was used to isolate cDNAs encoding NR4 from WEHI-3B cell, peritoneal macrophage, bone marrow, skin and kidney libraries. A composite of the nucleotide sequence [SEQ ID NO:1] and predicted amino acid sequence [SEQ ID NO:2] of these cDNAs is shown in Figure 1. The NR4 cDNA is predicted to encode for a protein of 424 amino acid residues, containing a putative signal
15 sequence and transmembrane domain. The extracellular region of the protein containing a putative signal sequence and transmembrane domain. The extracellular region of the protein contained an immunoglobulin-like domain (amino acids 27-117), in addition to a typical haemopoietin receptor domain (amino acids 118-340) which includes four conserved cysteine residues and the characteristic Trp-Ser-Asp-Trp-Ser [SEQ ID NO:3] motif (Figure 1). The
20 cytoplasmic tail of the new receptor was 60 amino acids in length.

EXAMPLE 7

Expression pattern of NR4 cDNA

The pattern of NR4 mRNA expression was examined by Northern analyses. Two hybridising
25 species of 5.2 and 2.2 kb in length were detected in mRNA from most tissues (Figure 2). NR4 mRNA was not detectable in skeletal muscle (Figure 2). Figure 8 shows expression of NR4 in mouse tissues.

- 17 -

EXAMPLE 8**NR4 encodes the IL-13 receptor α -chain (IL-13R α) - a specific binding subunit of the IL-13 receptor**

The apparent molecular weight is from about 50,000 to about 70,000 daltons and more particularly about 55,000 to about 65,000 daltons for NR4 expressed in COS cells estimated from Western blots using an anti-FLAG antibody, suggested that NR4 might encode the binding subunit of the IL-13 receptor. In order to test this possibility NR4 was expressed in COS cells. Untransfected COS cells expressed relatively low levels of IL-4 and IL-13 receptors. Upon transfection with a plasmid containing the NR4 cDNA, the number of IL-13 receptors but not IL-4 receptors expressed by COS cells was dramatically increased (Figure 3A; 100,000 to 500,000 receptors per cell). The affinity of IL-13 for NR4 expressed by COS cells was low ($K_D \sim 2$ -10 nM) and binding was specific since it was in competition with unlabelled IL-13 but not other cytokines including IL-2, IL-4, IL-7, IL-9 or IL-15 (Figure 4A). These results suggest that NR4 is the IL-13 receptor α -chain (IL-13R α).

15

EXAMPLE 9**The IL-13R α (NR4) and the IL-4R α are shared components of the IL-4 and IL-3 receptors**

In order to investigate the relationship between IL-4 and IL-13 receptors, the IL-4 responsive cell line CTLL was examined. Parental CTLL cells expressed a single class of IL-4 receptor ($K_D \sim 660$ pM; ~ 3600 receptors per cell) but no detectable IL-13 receptors (Figure 3B). The IL-4 receptors expressed by CTLL cells appeared to be specific since binding of ^{125}I -IL-4 was in competition with unlabelled IL-4 but not IL-13 (Figure 4B). Upon expression of the IL-13R α (NR4) in CTLL cells no change was observed in the number or affinity of IL-4 receptors, while a single class of high affinity IL-13 receptors was detected (Figure 3C; $K_D \sim 75$ pM; 1350 receptors per cell). The affinity of IL-13 for the IL-13R α (NR4) expressed in CTLL cells was higher than in COS cells, suggesting that the former expressed a protein capable of interacting with the IL-13R α (NR4) to increase the affinity for IL-13. A likely candidate based on previous studies is the IL-4R α . In order to explore this possibility the ability of IL-4 to compete with ^{125}I -IL-13 for binding to CTLL cells expressing the IL-13R α (NR4) was assessed.

30

- 18 -

Figure 4B shows that IL-4 and IL-13 were equally effective in competing for ^{125}I -IL-13 binding ($\text{IC}_{50} \sim 300\text{pM}$; Figure 4C) and, in addition, were able to compete with ^{125}I -IL-4 for binding ($\text{IC}_{50} \sim 300\text{ pm}$; Figure 4D).

5

EXAMPLE 10

Expression of the IL-13R α (NR4) is necessary for transduction of a proliferative signal by IL-13

CTLL cells require the addition of exogenous cytokines for survival and proliferation. IL-2 was found to be a potent proliferative stimulus for CTLL cells ($\text{EC}_{50} \sim 100\text{-}200\text{ pM}$), while IL-4 was relatively weak ($\text{EC}_{50} 2\text{-}7\text{ nM}$) and IL-13 was inactive (Figure 5A). Expression of the IL-13R α (NR4) in CTLL cells resulted in the ability to survive and proliferate weakly in response to IL-13 ($\text{EC}_{50} \sim 700\text{ pM}$) and to proliferate somewhat more strongly than parental cells in response to IL-4 ($\text{EC}_{50} \sim 700\text{ pM}$; Figure 5B).

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EXAMPLE 11

Cloning of Human IL-13R α (NR4)

In order to determine whether genes homologous to murine IL-13R α (NR4) exist in other vertebrate species, a probe encompassing nucleotides 840 to 1270 of murine IL-13R α (NR4) was hybridised to *Eco*RI digested genomic DNA from various species. Hybridisation was carried out in 500 mM Na_2HPO_4 ($\sim 5\times\text{SSC}$) at 50°C overnight. The filter was washed in 40 mM Na_2HPO_4 ($\sim 0.2\times\text{SSC}$) at 50°C for 2 hours and exposed to autoradiographic film for 48 hours. Figure 6 illustrates that relatively few (1 to 5) hybridising bands are observed in genomic DNA from various species, including human. This suggests that it is feasible to clone human IL-13R α (NR4) using a murine cDNA probe. A human bone marrow cDNA library clones in the λZAPII bacteriophage was therefore screened with two probes (nucleotides 82-840 and 840 to 1270) from the murine IL-13R α (NR4) cDNA. Hybridisation was carried out overnight in $6\times\text{SSC}$, 0.1% w/v SDS at 42°C . Filters were washed at $2\times\text{SSC}$, 0.1% w/v SDS at 50°C for 2 hours and exposed for 48 hours to autoradiographic film. Plaques that hybridised to both murine IL-13R α (NR4) probes were picked and purified in the conventional manner. The cDNA inserts from the hybridising bacteriophage were excised into the pBluescript plasmid and

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- 19 -

sequenced in their entirety using an ABI automated sequencer. Figure 7 shows a composite of the sequence of the clones isolated and reveals that the clones encode a protein that shares a high degree of sequence similarity with murine IL-13R α (NR4). The clones encode for the entire mature coding region of the protein, but lack the initiation methionine and the signal sequence; the high degree of sequence similarity (311/401 amino acids ~ 78%) predicates that this cDNA is the human homologue of the murine IL-13R α (NR4).

EXAMPLE 12

Soluble Murine IL-13R α (NR4)

10 Constructs were engineered to express soluble versions of NR4 with an N-terminal "FLAG" epitope (International Biotechnologies/Eastman Kodak, New Haven CT). First, a derivative of the mammalian expression vector pEF-BOS was generated so that it contained DNA encoding the signal sequence of murine IL-3 (MVLASSTTSIHTMLLLLLMLFHLGLQASIS [SEQ ID NO:5]) and the FLAG epitope (DYKDDDDK [SEQ ID NO:6]), followed by a unique
15 XbaI cloning site. This vector was named pEF/IL3SIG/FLAG. The mature extracellular part of the NR4 coding region (Thr27 to Thr344) was generated by PCR using primers 1478 and 1480. The resulting product was digested with XbaI and was cloned into the XbaI site of pEF/IL3SIG/FLAG to give pEF/IL3SIG/FLAG/sol NR4. The identity of the construct was confirmed by dideoxy sequencing. OLIGO 1478 5'
20 AGCTTCTAGAACAGAAAGTTCAGCCACCTGTG 3' [SEQ ID NO:7]; OLIGO 1480 5' AACTCCACCTTCTACACCACCTGATCTAGA 3' [SEQ ID NO:8].

After transfection into CHO cells, expressed, soluble NR4 was purified from CHO cell-conditioned medium on an anti-FLAG antibody (M2) affinity column by elution with free
25 FLAG peptide (Science Imaging Systems).

Consistent with the low affinity of IL-13 for NR4 expressed by COS cells, purified soluble NR4 appeared unable to bind IL-13 as assessed by gel filtration chromatography. However, using sensitive cross-linking assays, the ability of soluble IL-13R α (NR4) to bind IL-13 was
30 demonstrated (Figure 9, lane 1). This interaction was competed for by unlabelled IL-13 but not

- 20 -

by unlabelled IL-4 (Figure 9, lanes 2 and 3).

EXAMPLE 13

A Polyclonal Antisera to Soluble IL-13R α (NR4)

5 A polyclonal antiserum to NR4 was prepared by injecting purified soluble NR4 into rabbits which were bled after 3 months. This antisera immunoprecipitated the cross-linked product of 125 I-IL-13 with soluble NR4 (Figure 10, lane 11) while no immunoprecipitation was observed with pre-immune serum (Figure 10, lane 9). Immunoprecipitation of the complex was not inhibited by the FLAG peptide (Figure 10, lane 10).

10

The immunoprecipitation assay was conducted as follows:

The cross-linking reactions were terminated by the addition of Tris-HCl, pH 7.5, to a final concentration of 40 mM. The samples were then mixed with 1:50 diluted control rabbit serum
15 or anti-NR4 serum which had been pre-incubated with or without FLAG peptide. After incubation for 30 min at 4°C, the mixtures were added to 40 μ l of 50% v/v protein G-Sepharose gel slurry (Pharmacia) and incubated for 30 min at 4°C. The samples were centrifuged and the protein G beads were washed 3 x 0.5 ml PBS, mixed with 40 μ l of 2X concentrated SDS-PAGE sample buffer and heated for 2 min at 95°C. The supernatants were
20 analysed by 13% w/v SDS-PAGE under non-reducing conditions.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of
25 the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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- 22 -

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- 23 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE WALTER AND ELIZA HALL INSTITUTE OF
MEDICAL RESEARCH

(ii) TITLE OF INVENTION: A NOVEL HAEMOPOIETIN RECEPTOR AND
GENETIC SEQUENCES ENCODING SAME - III

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: DAVIES COLLISON CAVE

(B) STREET: 1 LITTLE COLLINS STREET

(C) CITY: MELBOURNE

(D) STATE: VICTORIA

(E) COUNTRY: AUSTRALIA

(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: AU PROVISIONAL

(B) FILING DATE: 09-SEP-1996

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PN6135/95

(B) FILING DATE: 23-OCT-1995

(A) APPLICATION NUMBER: PN7276/95

(B) FILING DATE: 22-DEC-1995

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: HUGHES DR, E JOHN L

(C) REFERENCE/DOCKET NUMBER: EJH/EK

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +61 3 9254 2777

(B) TELEFAX: +61 3 9254 2770

- 24 -

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1680 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1272

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGAAAAGATA GAATAAATGG CCTCGTGCCG AATTCGGCAC GAGCCGAGGC GAGGGCCTGC	-1
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ACC GCC ACC GTG GGC CAA GTT GCC GCG GCC ACA GAA GTT CAG CCA CCT	96
Thr Ala Thr Val Gly Gln Val Ala Ala Ala Thr Glu Val Gln Pro Pro	
20 25 30	
GTG ACG AAT TTG AGC GTC TCT GTC GAA AAT CTC TGC ACG ATA ATA TGG	144
Val Thr Asn Leu Ser Val Ser Val Glu Asn Leu Cys Thr Ile Ile Trp	
35 40 45	
ACG TGG AGT CCT CCT GAA GGA GCC AGT CCA AAT TGC ACT CTC AGA TAT	192
Thr Trp Ser Pro Pro Glu Gly Ala Ser Pro Asn Cys Thr Leu Arg Tyr	
50 55 60	
TTT AGT CAC TTT GAT GAC CAA CAG GAT AAG AAA ATT GCT CCA GAA ACT	240
Phe Ser His Phe Asp Asp Gln Gln Asp Lys Lys Ile Ala Pro Glu Thr	
65 70 75 80	
CAT CGT AAA GAG GAA TTA CCC CTG GAT GAG AAA ATC TGT CTG CAG GTG	288
His Arg Lys Glu Glu Leu Pro Leu Asp Glu Lys Ile Cys Leu Gln Val	
85 90 95	
GGC TCT CAG TGT AGT GCC AAT GAA AGT GAG AAG CCT AGC CCT TTG GTG	336
Gly Ser Gln Cys Ser Ala Asn Glu Ser Glu Lys Pro Ser Pro Leu Val	
100 105 110	
AAA AAG TGC ATC TCA CCC CCT GAA GGT GAT CCT GAG TCC GCT GTG ACT	384
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130 135 140	

- 25 -

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180 185 190	
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Gln Asn Ser Thr Phe Tyr Thr Thr Met Leu Leu Thr Ile Pro Val Phe	
340 345 350	
GTC GCA GTG GCA GTC ATA ATC CTC CTT TTT TAC CTG AAA AGG CTT AAG	1104
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355 360 365	

- 26 -

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Met Phe Gly Asp Gln Asn Asp Asp Thr Leu His Trp Lys Lys Tyr Asp	
385 390 395 400	
ATC TAT GAG AAA CAA TCC AAA GAA GAA ACG GAT TCT GTA GTG CTG ATA	1248
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405 410 415	
GAA AAC CTG AAG AAA GCA GCT CCT TGATGGGGAG AAGTGATTTC TTTCTTGCCT	1302
Glu Asn Leu Lys Lys Ala Ala Pro	
420	
TCAATGTGAC CCTGTGAAGA TTTATTGCAT TCTCCATTTG TTATCTGGGG GACTTGTTAA	1362
ATAGAAACTG AAACACTCT TGAAAAACAG GCAGCTCCTA AGAGCCACAG GTCTTGATGT	1422
GACTTTTGCA TTGAAAACCC AAACCCAAAG GAGCTCCTTC CAAGAAAAGC AAGAGTTCTT	1482
CTCGTTCCTT GTTCCAATCC CTAAGAGCAG ATGTTTTGCC AAATCCCCAA ACTAGAGGAC	1542
AAAGACAAGG GGACAATGAC CATCAATTCA TCTAATCAGG AATTGTGATG GCTTCCTAAG	1602
GAATCTCTGC TTGCTCTG	1620

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 424 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Arg Pro Ala Leu Leu Gly Glu Leu Leu Val Leu Leu Leu Trp
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20 25 30
Val Thr Asn Leu Ser Val Ser Val Glu Asn Leu Cys Thr Ile Ile Trp
35 40 45
Thr Trp Ser Pro Pro Glu Gly Ala Ser Pro Asn Cys Thr Leu Arg Tyr
50 55 60

- 27 -

Phe	Ser	His	Phe	Asp	Asp	Gln	Gln	Asp	Lys	Lys	Ile	Ala	Pro	Glu	Thr	65	70	75	80
His	Arg	Lys	Glu	Glu	Leu	Pro	Leu	Asp	Glu	Lys	Ile	Cys	Leu	Gln	Val	85	90	95	
Gly	Ser	Gln	Cys	Ser	Ala	Asn	Glu	Ser	Glu	Lys	Pro	Ser	Pro	Leu	Val	100	105	110	
Lys	Lys	Cys	Ile	Ser	Pro	Pro	Glu	Gly	Asp	Pro	Glu	Ser	Ala	Val	Thr	115	120	125	
Glu	Leu	Lys	Cys	Ile	Trp	His	Asn	Leu	Ser	Tyr	Met	Lys	Cys	Ser	Trp	130	135	140	
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Trp	Tyr	Ser	Ser	Leu	Glu	Lys	Ser	Arg	Gln	Cys	Glu	Asn	Ile	Tyr	Arg	165	170	175	
Glu	Gly	Gln	His	Ile	Ala	Cys	Ser	Phe	Lys	Leu	Thr	Lys	Val	Glu	Pro	180	185	190	
Ser	Phe	Glu	His	Gln	Asn	Val	Gln	Ile	Met	Val	Lys	Asp	Asn	Ala	Gly	195	200	205	
Lys	Ile	Arg	Pro	Ser	Cys	Lys	Ile	Val	Ser	Leu	Thr	Ser	Tyr	Val	Lys	210	215	220	
Pro	Asp	Pro	Pro	His	Ile	Lys	His	Leu	Leu	Leu	Lys	Asn	Gly	Ala	Leu	225	230	235	240
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Glu	Val	Glu	Glu	Asp	Lys	Cys	Gln	Asn	Ser	Glu	Ser	Asp	Arg	Asn	Met	275	280	285	
Glu	Gly	Thr	Ser	Cys	Phe	Gln	Leu	Pro	Gly	Val	Leu	Ala	Asp	Ala	Val	290	295	300	
Tyr	Thr	Val	Arg	Val	Arg	Val	Lys	Thr	Asn	Lys	Leu	Cys	Phe	Asp	Asp	305	310	315	320
Asn	Lys	Leu	Trp	Ser	Asp	Trp	Ser	Glu	Ala	Gln	Ser	Ile	Gly	Lys	Glu	325	330	335	
Gln	Asn	Ser	Thr	Phe	Tyr	Thr	Thr	Met	Leu	Leu	Thr	Ile	Pro	Val	Phe	340	345	350	

•

(2) INFORMATION FOR SEQ ID NO:3:

(A) LENGTH: 1248 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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			20					25					30			
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		35					40					45				
GAT	AAG	AAA	ATA	GCT	CCG	GAA	ACT	CGT	CGT	TCA	ATA	GAA	GTA	CCC	CTG	192
Asp	Lys	Lys	Ile	Ala	Pro	Glu	Thr	Arg	Arg	Ser	Ile	Glu	Val	Pro	Leu	
	50					55				60						
AAT	GAG	AGG	ATT	TGT	CTG	CAA	GTG	GGG	TCC	CAG	TGT	AGC	ACC	AAT	GAG	240
Asn	Glu	Arg	Ile	Cys	Leu	Gln	Val	Gly	Ser	Gln	Cys	Ser	Thr	Asn	Glu	
65					70				75						80	

- 29 -

AGT GAG AAG CCT AGC ATT TTG GTT GAA AAA TGC ATC TCA CCC CCA GAA	288
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85 90 95	
GGT GAT CCT GAG TCT GCT GTG ACT GAA CTT CAA TGC ATT TGG CAC AAC	336
Gly Asp Pro Glu Ser Ala Val Thr Glu Leu Gln Cys Ile Trp His Asn	
100 105 110	
CTG AGC TAC ATG AAG TGT TCT TGG CTC CCT GGA AGG AAT ACC AGT CCC	384
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115 120 125	
GAC ACT AAC TAT ACT CTC TAC TAT TGG CAC AGA AGC CTG GAA AAA ATT	432
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130 135 140	
CAT CAA TGT GAA AAC ATC TTT AGA GAA GGC CAA TAC TTT GGT TGT TCC	480
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145 150 155 160	
TTT GAT CTG ACC AAA GTG AAG GAT TCC AGT TTT GAA CAA CAC AGT GTC	528
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165 170 175	
CAA ATA ATG GTC AAG GAT AAT GCA GGA AAA ATT AAA CCA TCC TTC AAT	576
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180 185 190	
ATA GTG CCT TTA ACT TCC CGT GTG AAA CCT GAT CCT CCA CAT ATT AAA	624
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195 200 205	
AAC CTC TCC TTC CAC AAT GAT GAC CTA TAT GTG CAA TGG GAG AAT CCA	672
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210 215 220	
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Gln Asn Phe Ile Ser Arg Cys Leu Phe Tyr Glu Val Glu Val Asn Asn	
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275 280 285	
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Lys Thr Asn Lys Leu Cys Tyr Glu Asp Asp Lys Leu Trp Ser Asn Trp	
290 295 300	

- 31 -

Ser	Glu	Lys	Pro	Ser	Ile	Leu	Val	Glu	Lys	Cys	Ile	Ser	Pro	Pro	Glu	85	90	95
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Leu	Ser	Tyr	Met	Lys	Cys	Ser	Trp	Leu	Pro	Gly	Arg	Asn	Thr	Ser	Pro	115	120	125
Asp	Thr	Asn	Tyr	Thr	Leu	Tyr	Tyr	Trp	His	Arg	Ser	Leu	Glu	Lys	Ile	130	135	140
His	Gln	Cys	Glu	Asn	Ile	Phe	Arg	Glu	Gly	Gln	Tyr	Phe	Gly	Cys	Ser	145	150	155
Phe	Asp	Leu	Thr	Lys	Val	Lys	Asp	Ser	Ser	Phe	Glu	Gln	His	Ser	Val	165	170	175
Gln	Ile	Met	Val	Lys	Asp	Asn	Ala	Gly	Lys	Ile	Lys	Pro	Ser	Phe	Asn	180	185	190
Ile	Val	Pro	Leu	Thr	Ser	Arg	Val	Lys	Pro	Asp	Pro	Pro	His	Ile	Lys	195	200	205
Asn	Leu	Ser	Phe	His	Asn	Asp	Asp	Leu	Tyr	Val	Gln	Trp	Glu	Asn	Pro	210	215	220
Gln	Asn	Phe	Ile	Ser	Arg	Cys	Leu	Phe	Tyr	Glu	Val	Glu	Val	Asn	Asn	225	230	235
Ser	Gln	Thr	Glu	Thr	His	Asn	Val	Phe	Tyr	Val	Gln	Glu	Ala	Lys	Cys	245	250	255
Glu	Asn	Pro	Glu	Phe	Glu	Arg	Asn	Val	Glu	Asn	Thr	Ser	Cys	Phe	Met	260	265	270
Val	Pro	Gly	Val	Leu	Pro	Asp	Thr	Leu	Asn	Thr	Val	Arg	Ile	Arg	Val	275	280	285
Lys	Thr	Asn	Lys	Leu	Cys	Tyr	Glu	Asp	Asp	Lys	Leu	Trp	Ser	Asn	Trp	290	295	300
Ser	Gln	Glu	Met	Ser	Ile	Gly	Lys	Lys	Arg	Asn	Ser	Thr	Leu	Tyr	Ile	305	310	315
Thr	Met	Leu	Leu	Ile	Val	Pro	Val	Ile	Val	Ala	Gly	Ala	Ile	Ile	Val	325	330	335
Leu	Leu	Leu	Tyr	Leu	Lys	Arg	Leu	Lys	Ile	Ile	Ile	Phe	Pro	Pro	Ile	340	345	350
Pro	Asp	Pro	Gly	Lys	Ile	Phe	Lys	Glu	Met	Phe	Gly	Asp	Gln	Asn	Asp	355	360	365

- 32 -

Asp Thr Leu His Trp Lys Lys Tyr Asp Ile Tyr Glu Lys Gln Thr Lys
370 375 380

Glu Glu Thr Asp Ser Val Val Leu Ile Glu Asn Leu Lys Lys Ala Ser
385 390 395 400

Gln

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Val Leu Ala Ser Ser Thr Thr Ser Ile His Thr Met Leu Leu Leu
5 10 15

Leu Leu Met Leu Phe His Leu Gly Leu Gln Ala Ser Ile Ser
20 25 30

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Asp Tyr Lys Asp Asp Asp Lys
5

- 33 -

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 31 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AGCTTCTAGA ACAGAAGTTC AGCCACCTGT G

31

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AACTCCACCT TCTACACCAC CTGATCTAGA

30

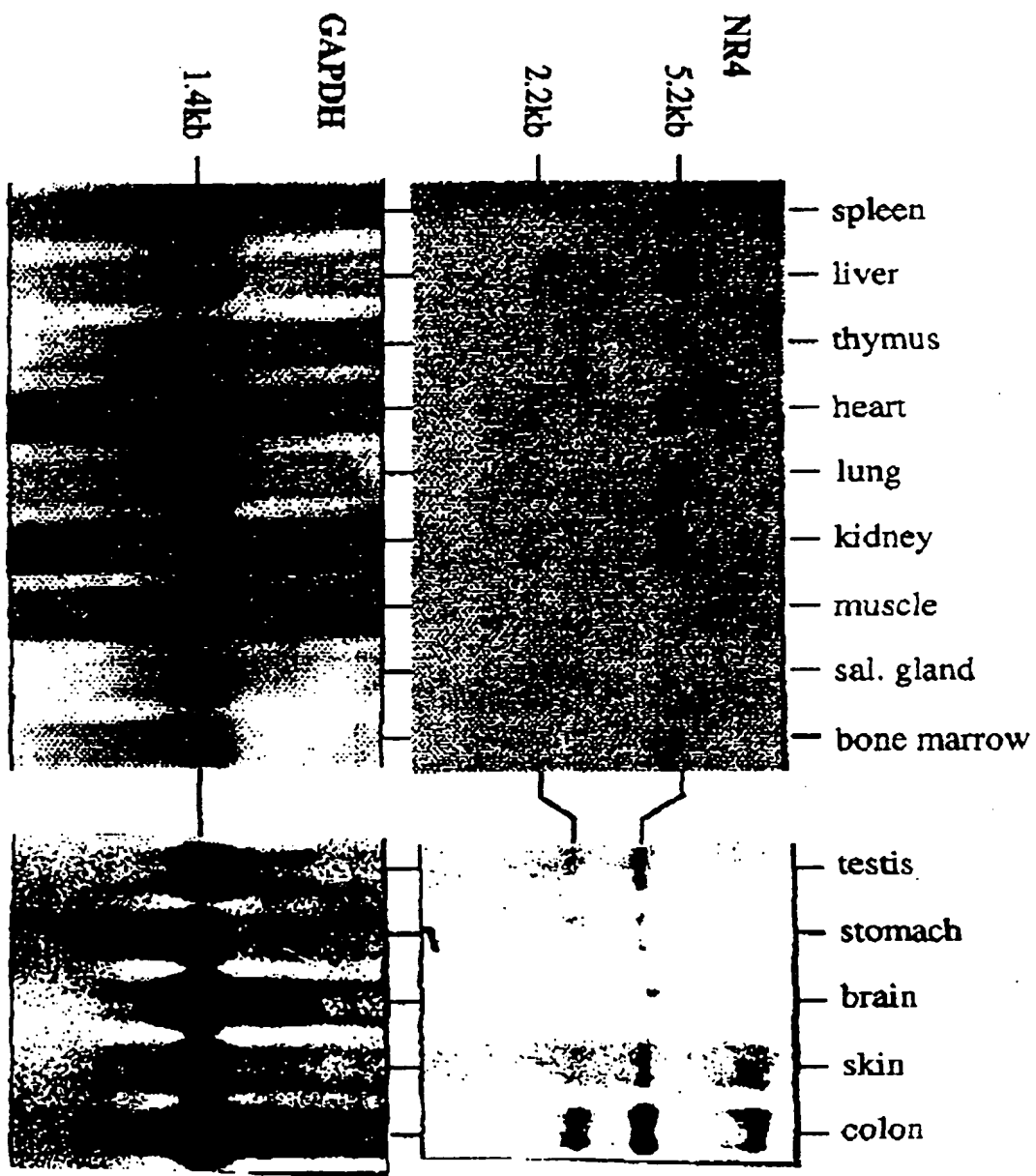
DATED this 9th day of September, 1996

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DAVIES COLLISON CAVE

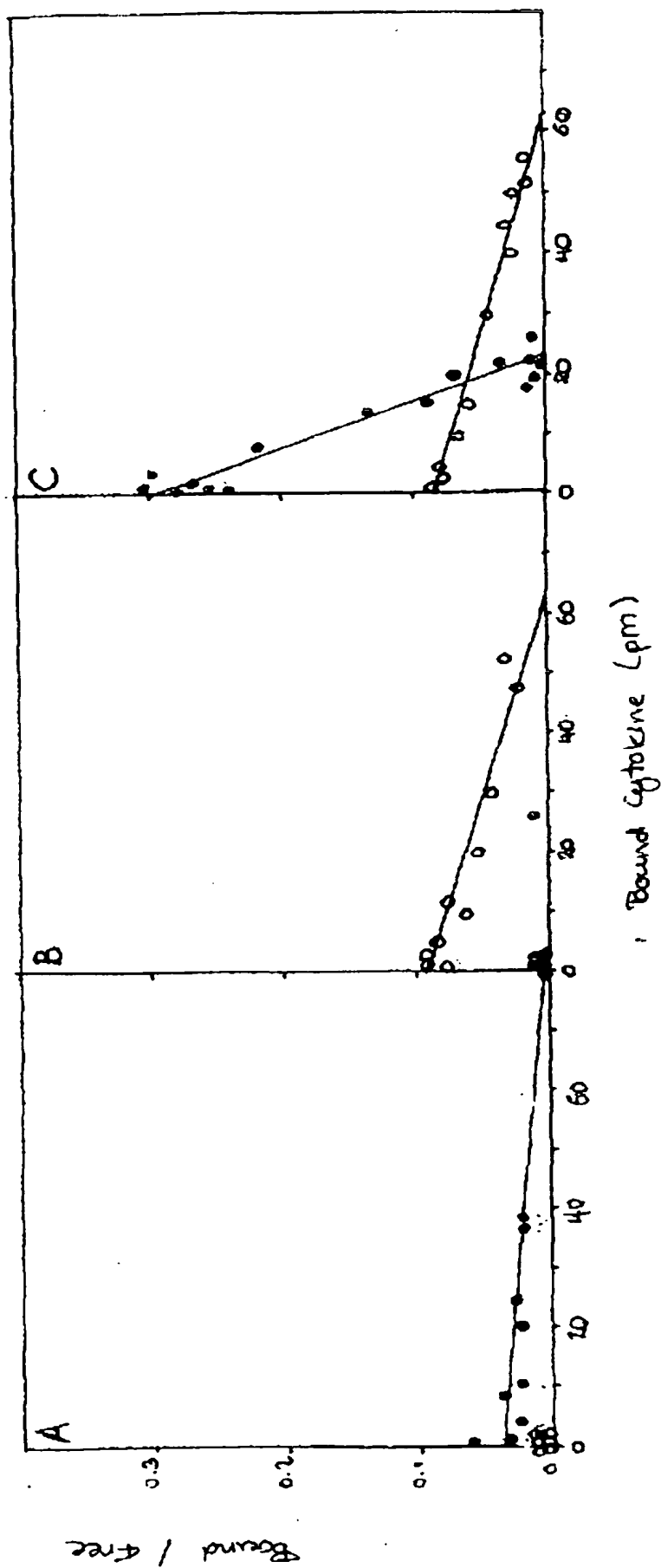
1 123456789101112131415161718192021222324252627282930313233343536373839404142434445464748495051525354555657585960616263646566676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107108109110111112113114115116117118119120121122123124125126127128129130131132133134135136137138139140141142143144145146147148149150151152153154155156157158159160161162163164165166167168169170171172173174175176177178179180181182183184185186187188189190191192193194195196197198199200201202203204205206207208209210211212213214215216217218219220221222223224225226227228229230231232233234235236237238239240241242243244245246247248249250251252253254255256257258259260261262263264265266267268269270271272273274275276277278279280281282283284285286287288289290291292293294295296297298299300301302303304305306307308309310311312313314315316317318319320321322323324325326327328329330331332333334335336337338339340341342343344345346347348349350351352353354355356357358359360361362363364365366367368369370371372373374375376377378379380381382383384385386387388389390391392393394395396397398399400401402403404405406407408409410411412413414415416417418419420421422423424425426427428429430431432433434435436437438439440441442443444445446447448449450451452453454455456457458459460461462463464465466467468469470471472473474475476477478479480481482483484485486487488489490491492493494495496497498499500501502503504505506507508509510511512513514515516517518519520521522523524525526527528529530531532533534535536537538539540541542543544545546547548549550551552553554555556557558559560561562563564565566567568569570571572573574575576577578579580581582583584585586587588589590591592593594595596597598599600601602603604605606607608609610611612613614615616617618619620621622623624625626627628629630631632633634635636637638639640641642643644645646647648649650651652653654655656657658659660661662663664665666667668669670671672673674675676677678679680681682683684685686687688689690691692693694695696697698699700701702703704705706707708709710711712713714715716717718719720721722723724725726727728729730731732733734735736737738739740741742743744745746747748749750751752753754755756757758759760761762763764765766767768769770771772773774775776777778779780781782783784785786787788789790791792793794795796797798799800801802803804805806807808809810811812813814815816817818819820821822823824825826827828829830831832833834835836837838839840841842843844845846847848849850851852853854855856857858859860861862863864865866867868869870871872873874875876877878879880881882883884885886887888889890891892893894895896897898899900901902903904905906907908909910911912913914915916917918919920921922923924925926927928929930931932933934935936937938939940941942943944945946947948949950951952953954955956957958959960961962963964965966967968969970971972973974975976977978979980981982983984985986987988989990991992993994995996997998999100010011002100310041005100610071008100910101011101210131014101510161017101810191020102110221023102410251026102710281029103010311032103310341035103610371038103910401041104210431044104510461047104810491050105110521053105410551056105710581059106010611062106310641065106610671068106910701071107210731074107510761077107810791080108110821083108410851086108710881089109010911092109310941095109610971098109911001100111002110031100411005110061100711008110091101011011110121101311014110151101611017110181101911020110211102211023110241102511026110271102811029110301103111032110331103411035110361103711038110391104011041110421104311044110451104611047110481104911050110511105211053110541105511056110571105811059110601106111062110631106411065110661106711068110691107011071110721107311074110751107611077110781107911080110811108211083110841108511086110871108811089110901109111092110931109411095110961109711098110991110011100111100211100311100411100511100611100711100811100911101011101111101211101311101411101511101611101711101811101911102011102111102211102311102411102511102611102711102811102911103011103111103211103311103411103511103611103711103811103911104011104111104211104311104411104511104611104711104811104911105011105111105211105311105411105511105611105711105811105911106011106111106211106311106411106511106611106711106811106911107011107111107211107311107411107511107611107711107811107911108011108111108211108311108411108511108611108711108811108911109011109111109211109311109411109511109611109711109811109911110011110011111002111100311110041111005111100611110071111008111100911110101111011111101211110131111014111101511110161111017111101811110191111020111102111110221111023111102411110251111026111102711110281111029111103011110311111032111103311110341111035111103611110371111038111103911110401111041111104211110431111044111104511110461111047111104811110491111050111105111110521111053111105411110551111056111105711110581111059111106011110611111062111106311110641111065111106611110671111068111106911110701111071111107211110731111074111107511110761111077111107811110791111080111108111110821111083111108411110851111086111108711110881111089111109011110911111092111109311110941111095111109611110971111098111109911111001111100111111002111110031111100411111005111110061111100711111008111110091111101011111011111101211111013111110141111101511111016111110171111101811111019111110201111102111111022111110231111102411111025111110261111102711111028111110291111103011111031111110321111103311111034111110351111103611111037111110381111103911111040111110411111104211111043111110441111104511111046111110471111104811111049111110501111105111111052111110531111105411111055111110561111105711111058111110591111106011111061111110621111106311111064111110651111106611111067111110681111106911111070111110711111107211111073111110741111107511111076111110771111107811111079111110801111108111111082111110831111108411111085111110861111108711111088111110891111109011111091111110921111109311111094111110951111109611111097111110981111109911111100111111001111111002111111003111111004111111005111111006111111007111111008111111009111111010111111011111110121111110131111110141111110151111110161111110171111110181111110191111110201111110211111110221111110231111110241111110251111110261111110271111110281111110291111110301111110311111110321111110331111110341111110351111110361111110371111110381111110391111110401111110411111110421111110431111110441111110451111110461111110471111110481111110491111110501111110511111110521111110531111110541111110551111110561111110571111110581111110591111110601111110611111110621111110631111110641111110651111110661111110671111110681111110691111110701111110711111110721111110731111110741111110751111110761111110771111110781111110791111110801111110811111110821111110831111110841111110851111110861111110871111110881111110891111110901111110911111110921111110931111110941111110951111110961111110971111110981111110991111111001111111001111111100211111110031111111004111111100511111110061111111007111111100811111110091111111010111111101111111101211111110131111111014111111101511111110161111111017111111101811111110191111111020111111102111111110221111111023111111102411111110251111111026111111102711111110281111111029111111103011111110311111111032111111103311111110341111111035111111103611111110371111111038111111103911111110401111111041111111104211111110431111111044111111104511111110461111111047111111104811111110491111111050111111105111111110521111111053111111105411111110551111111056111111105711111110581111111059111111106011111110611111111062111111106311111110641111111065111111106611111110671111111068111111106911111110701111111071111111107211111110731111111074111111107511111110761111111077111111107811111110791111111080111111108111111110821111111083111111108411111110851111111086111111108711111110881111111089111111109011111110911111111092111111109311111110941111111095111111109611111110971111111098111111109911111111001111111100111111111002111111110031111111100411111111005111111110061111111100711111111008111111110091111111101011111111011111111101211111111013111111110141111111101511111111016111111110171111111101811111111019111111110201111111102111111111022111111110231111111102411111111025111111110261111111102711111111028111111110291111111103011111111031111111110321111111103311111111034111111110351111111103611111111037111111110381111111103911111111040111111110411111111104211111111043111111110441111111104511111111046111111110471111111104811111111049111111110501111111105111111111052111111110531111111105411111111055111111110561111111105711111111058111111110591111111106011111111061111111110621111111106311111111064111111110651111111106611111111067111111110681111111106911111111070111111110711111111107211111111073111111110741111111107511111111076111111110771111111107811111111079111111110801111111108111111111082111111110831111111108411111111085111111110861111111108711111111088111111110891111111109011111111091111111110921111111109311111111094111111110951111111109611111111097111111110981111111109911111111100111111111001111111111002111111111003111111111004111111111005111111111006111111111007111111111008111111111009111111111010111111111011111111110121111111110131111111110141111111110151111111110161111111110171111111110181111111110191111111110201111111110211111111110221111111110231111111110241111111110251111111110261111111110271111111110281111111110291111111110301111111110311111111110321111111110331111111110341111111110351111111110361111111110371111111110381111111110391111111110401111111110411111111110421111111110431111111110441111111110451111111110461111111110471111111110481111111110491111111110501111111110511111111110521111111110531111111110541111111110551111111110561111111110571111111110581111111110591111111110601111111110611111111110621111111110631111111110641111111110651111111110661111111110671111111110681111111110691111111110701111111110711111111110721111111110731111111110741111111110751111111110761111111110771111111110781111111110791111111110801111111110811111111110821111111110831111111110841111111110851111111110861111111110871111111110881111111110891111111110901111111110911111111110921111111110931111111110941111111110951111111110961111111110971111111110981111111110991111111111001111111111001111111111100211111111110031111111111004111111111100511111111110061111111111007111111111100811111111110091111111111010111111111101111111111101211111111110131111111111014111111111101511111111110161111111111017111111111101811111111110191111111111020111111111102111111111110221111111111023111111111102411111111110251111111111026111111111102711111111110281111111111029111111111103011111111110311111111111032111111111103311111111110341111111111035111111111103611111111110371111111111038111111111103911111111110401111111111041111111111104211111111110431111111111044111111111104511111111110461111111111047111111111104811111111110491111111111050111111111105111111111110521111111111053111111111105411111111110551111111111056111111111105711111111110581111111111059111111111106011111111110611111111111062111111111106311111111110641111111111065111111111106611111111110671111111111068111111111106911111111110701111111111071111111111107211111111110731111111111074111111111107511111111110761111111111077111111111107811111111110791111111111080111111111108111111111110821111111111083111111111108411111111110851111111111086111111111108711111111110881111111111089111111111109011111111110911111111111092111111111109311111111110941111111111095111111111109611111111110971111111111098111111111109911111111111001111111111100111111111111002111111111110031111111111100411111111111005111111111110061111111111100711111111111008111111111110091111111111101011111111111011111111111101211111111111013111111111110141111111111101511111111111016111111111110171111111111101811111111111019111111111110201111111111102111111111111022111111111110231111111111102411111111111025111111111110261111111111102711111111111028111111111110291111111111103011111111111031111111111110321111111111103311111111111034111111111110351111111111103611111111111037111111111110381111111111103911111111111040111111111110411111111111104211111111111043111111111110441111111111104511111111111046111111111110471111111111104811111111111049111111111110501111111111105111111111111052111111111110531111111111105411111111111055111111111110561111111111105711111111111058111111111110591111111111106011111111111061111111111110621111111111106311111111111064111111111110651111111111106611111111111067111111111110681111111111106



NRA Expression in various tissues

FIGURE 2

FIGURE 3



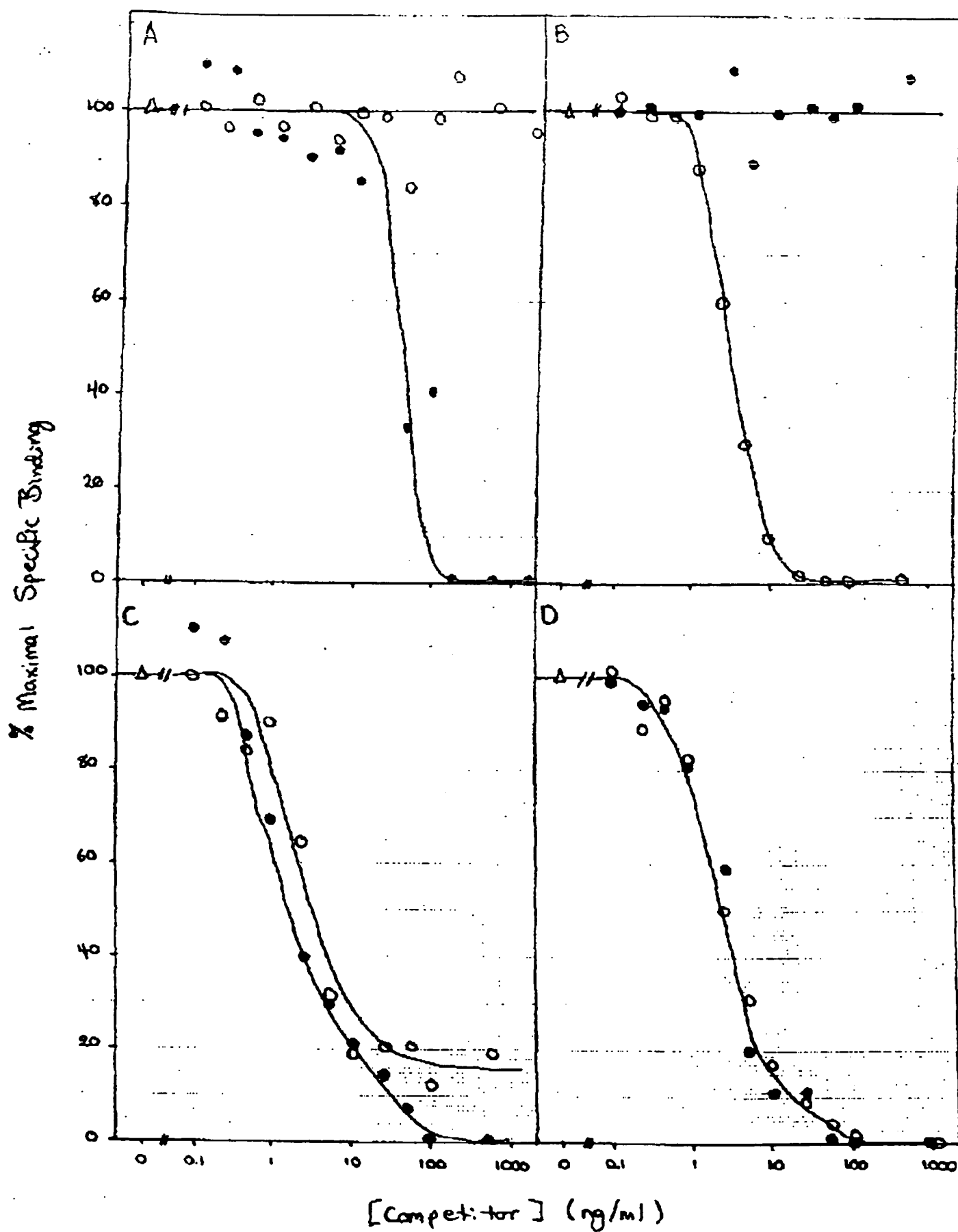


FIGURE 4

% Maximum Number of Viable Cells.

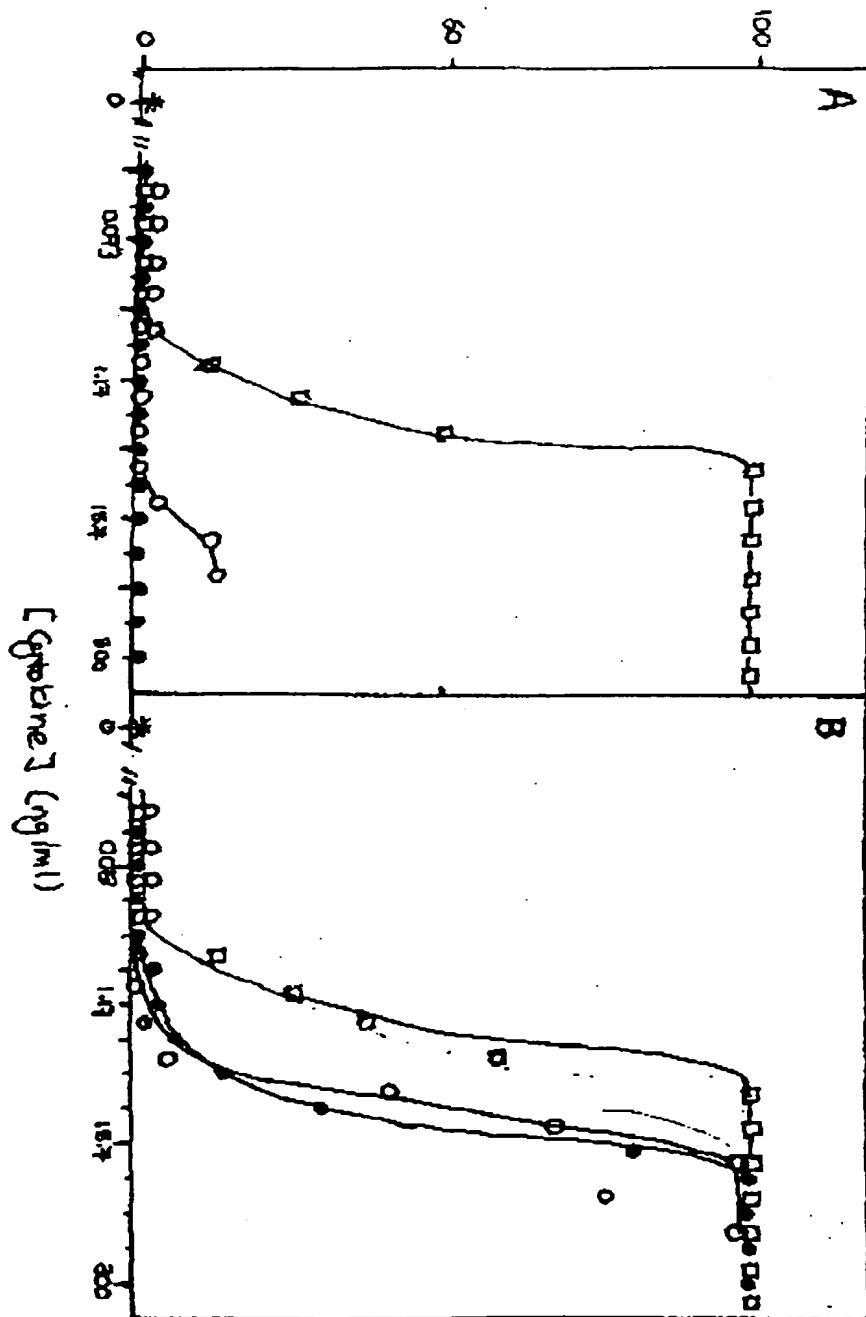


FIGURE 5

FIGURE 6

Cross-species conservation of the NR-4 (IL-13R α) gene

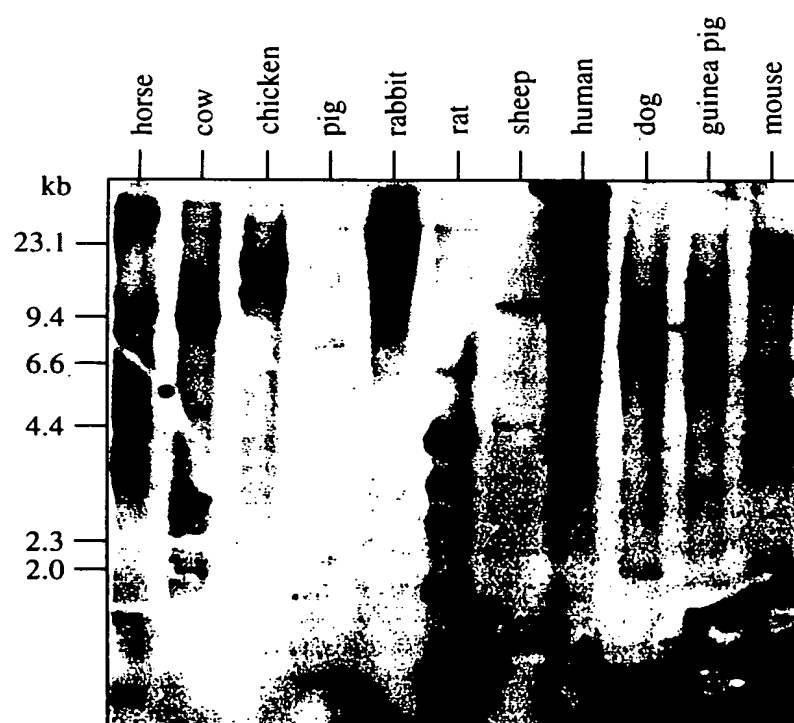


FIGURE 7

```

M  -60  tgaaaagatagaataaatggcctcgtgccgaattcgggcacgagccgagggcgagggcctgc

M  1    ATGGCGCGGCCAGCGCTGCTGGGCGAGCTGTTGGTGCTGCTACTGTGGACCGCCACCGTG
M  1    M A R P A L L G E L L V L L L W T A T V

H          A P T E T Q P P V T N L S V S V
H          GCGCCTACGGAAACTCAGCCACCTGTGACAAATTTGAGTGTCTCTGTT
          * * * * *
M  61    GGCCAAGTTGCCGCGGCCACAGAAGTTCAGCCACCTGTGACGAATTTGAGCGTCTCTGTC
M  21    G Q V A A A T E V Q P P V T N L S V S V

H          E N L C T V I W T W N P P E G A S S N C
H          GAAAACCTCTGCACAGTAATATGGACATGGAATCCACCCGAGGGAGCCAGCTCAAATTGT
          * * * * *
M  121   GAAAATCTCTGCACGATAATATGGACGTGGAGTCCTCCTGAAGGAGCCAGTCCAAATTGC
M  41    E N L C T I I W T W S P P E G A S P N C

H          S L W Y F S H F G D K Q D K K I A P E T
H          AGTCTATGGTATTTTAGTCAATTTGGCGACAAACAAGATAAGAAAATAGCTCCGGAAACT
          * * * * *
M  181   ACTCTCAGATATTTTAGTCACTTTGATGACCAACAGGATAAGAAAATTGCTCCAGAAACT
M  61    T L R Y F S H F D D Q Q D K K I A P E T

H          R R S I E V P L N E R I C L Q V G S Q C
H          CGTCGTTCAATAGAAGTACCCCTGAATGAGAGGATTTGTCTGCAAGTGGGGTCCCAGTGT
          * * * * *
M  241   CATCGTAAAGAGGAATTACCCCTGGATGAGAAAATCTGTCTGCAGGTGGGCTCTCAGTGT
M  81    H R K E E L P L D E K I C L Q V G S Q C

H          S T N E S E K P S I L V E K C I S P P E
H          AGCACCAATGAGAGTGAGAAGCCTAGCATTTTGGTTGAAAAATGCATCTCACCCCAGAA
          * * * * *
M  301   AGTGCCAATGAAAGTGAGAAGCCTAGCCCTTTGGTGAAAAAGTGCATCTCACCCCCTGAA
M  101   S A N E S E K P S P L V K K C I S P P E

H          G D P E S A V T E L Q C I W H N L S Y M
H          GGTGATCCTGAGTCTGCTGTGACTGAACTTCAATGCATTTGGCACAACCTGAGCTACATG
          * * * * *
M  361   GGTGATCCTGAGTCCGCTGTGACTGAGCTCAAGTGCATTTGGCATAACCTGAGCTATATG
M  121   G D P E S A V T E L K C I W H N L S Y M

H          K C S W L P G R N T S P D T N Y T L Y Y
H          AAGTGTTCCTGGCTCCCTGGAAGGAATACCACTCCCGACACTAACTATACTCTCTACTAT
          * * * * *
M  421   AAGTGTTCCTGGCTCCCTGGAAGGAATACAAGCCCTGACACACACTATACTCTGTACTAT
M  141   K C S W L P G R N T S P D T H Y T L Y Y

```

FIGURE 7 (continued...)

H		W H R S L E K I H Q C E N I F R E G Q Y
H		TGGCACAGAAGCCTGGAAAAAATTCATCAATGTGAAAACATCTTTAGAGAAGGCCAATAC
		* * * * *
M 481		TGGTACAGCAGCCTGGAGAAAAGTCGTCAATGTGAAAACATCTATAGAGAAGGTCAACAC
M 161		W Y S S L E K S R Q C E N I Y R E G Q H
H		F G C S F D L T K V K D S S F E Q H S V
H		TTTGGTTGTTTCCTTTGATCTGACCAAAGTGAAGGATTCCAGTTTTGAACAACACAGTGTC
		* * * * *
M 541		ATTGCTTGTTTCCTTTAAATTGACTAAAGTGGAACT---AGTTTTGAACATCAGAACGTT
M 181		I A C S F K L T K V E P - S F E H Q N V
H		Q I M V K D N A G K I K P S F N I V P L
H		CAAATAATGGTCAAGGATAATGCAGGAAAAATTAAACCATCCTTCAATATAGTGCCTTTA
		* * * * *
M 601		CAAATAATGGTCAAGGATAATGCTGGGAAAATTAGGCCATCCTGCAAAATAGTGTCTTTA
M 201		Q I M V K D N A G K I R P S C K I V S L
H		T S R V K P D P P H I K N L S F H N D D
H		ACTTCCCGTGTGAAACCTGATCCTCCACATATTTAAAACCTCTCCTTCCACAATGATGAC
		* * * * *
M 661		ACTTCTATGTGAAACCTGATCCTCCACATATTAACATCTTCTCCTCAAAAATGGTGCC
M 221		T S Y V K P D P P H I K H L L L K N G A
H		L Y V Q W E N P Q N F I S R C L F Y E V
H		CTATATGTGCAATGGGAGAATCCACAGAATTTTATTAGCAGATGCCTATTTTATGAAGTA
		* * * * *
M 721		TTATTAGTGCAGTGAAGAATCCACAAAATTTTAGAAGCAGATGCTTAACCTATGAAGTG
M 241		L L V Q W K N P Q N F R S R C L T Y E V
H		E V N N S Q T E T H N V F Y V Q E A K C
H		GAAGTCAATAACAGCCAAACTGAGACACATAATGTTTTCTACGTCCAAGAGGCTAAATGT
		* * * * *
M 781		GAGGTCAATAACTCAAACCGACCGACATAATTTTTAGAGGTTGAAGAGGACAAATGC
M 261		E V N N T Q T D R H N I L E V E E D K C
H		E N P E F E R N V E N T S C F M V P G V
H		GAGAATCCAGAATTTGAGAGAAATGTGGAGAATACATCTTGTTTCATGGTCCCTGGTGTT
		* * * * *
M 841		CAGAATTCCGAATCTGATAGAAACATGGAGGGTACAAGTTGTTTCCAACCTCCCTGGTGTT
M 281		Q N S E S D R N M E G T S C F Q L P G V
H		L P D T L N T V R I R V K T N K L C Y E
H		CTTCCTGATACTTTGAACACAGTCAGAATAAGAGTCAAACAAATAAGTTATGCTATGAG
		* * * * *
M 901		CTTGCCGACGCTGTCTACACAGTCAGAGTAAGAGTCAAACAAACAAGTTATGCTTTGAT
M 301		L A D A V Y T V R V R V K T N K L C F D
H		D D K L W S N W S Q E M S I G K K R N S
H		GATGACAAACTCTGGAGTAATTGGAGCCAAGAAATGAGTATAGGTAAGGAGCGCAATTC
		* * * * *
M 961		GACAACAAACTGTGGAGTGATTGGAGTGAAGCACAGAGTATAGGTAAGGAGCAAACTCC
M 321		D N K L W S D W S E A Q S I G K E Q N S

FIGURE 7 (continued...)

```

H      T L Y I T M L L I V P V I V A G A I I V
H      ACACTCTACATAACCATGTTACTCATTGTTCCAGTCATCGTCGCAGGTGCAATCATAGTA
      * * * * *
M 1021 ACCTTCTACACCACCATGTTACTCACCATTCCAGTCTTTGTCTGCAGTGGCAGTCATAATC
M 341   T F Y T T M L L T I P V F V A V A V I I

H      L L L Y L K R L K I I I F P P I P D P G
H      CTCCTGCTTTACCTAAAAAGGCTCAAGATTATTATATTCCTCCAATTCCTGATCCTGGC
      * * * * *
M 1081 CTCCTTTTTTACCTGAAAAGGCTTAAGATCATTATATTCCTCCAATTCCTGATCCTGGC
M 361   L L F Y L K R L K I I I F P P I P D P G

H      K I F K E M F G D Q N D D T L H W K K Y
H      AAGATTTTTTAAAGAAATGTTTGGAGACCAGAATGATGATACTCTGCACTGGAAGAAGTAC
      * * * * *
M 1141 AAGATTTTTTAAAGAAATGTTTGGAGACCAGAATGATGATACCCTGCACTGGAAGAAGTAT
M 381   K I F K E M F G D Q N D D T L H W K K Y

H      D I Y E K Q T K E E T D S V V L I E N L
H      GACATCTATGAGAAGCAAACCAAGGAGGAAACCGACTCTGTAGTGCTGATAGAAAACCTG
      * * * * *
M 1201 GACATCTATGAGAACAATCCAAAGAAGAAACGGATTCTGTAGTGCTGATAGAAAACCTG
M 401   D I Y E K Q S K E E T D S V V L I E N L

H      K K A S Q *
H      AAGAAAGCCTCTCAGTGAtggagataattttatttttaccttcactgtgaccttgagaaga
      * * *
M 1261 AAGAAAGCAGCTCCTTGAtggggagaagtgatttctttcttgaccttcaatgtgacctgt
M 421   K K A A P *

```

FIGURE 8

NR4 expression in mouse tissues

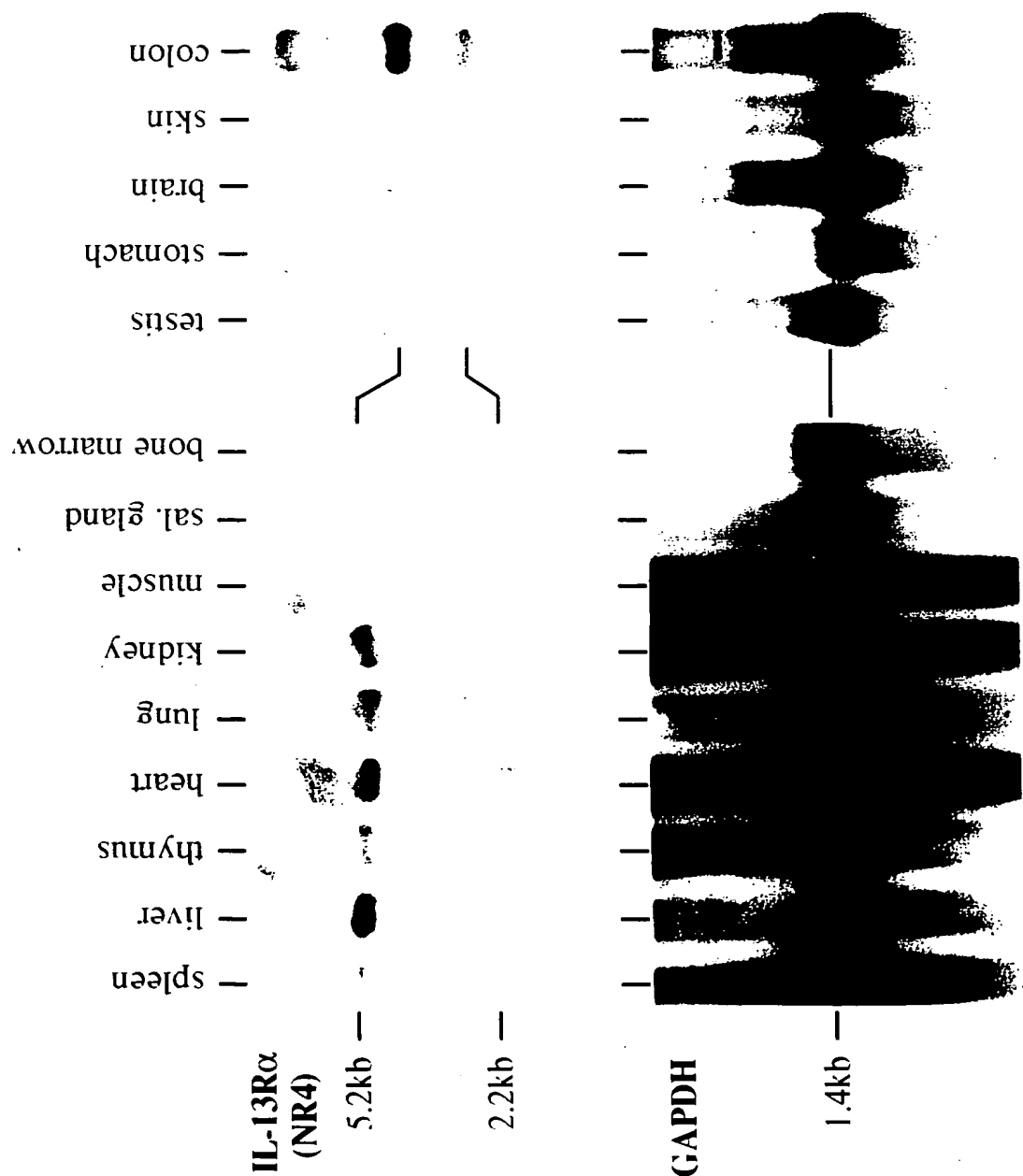


FIGURE 9

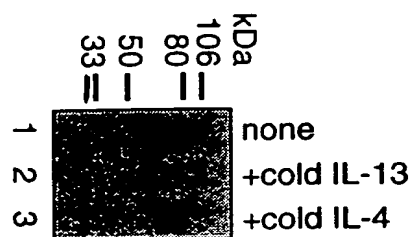


FIGURE 10

